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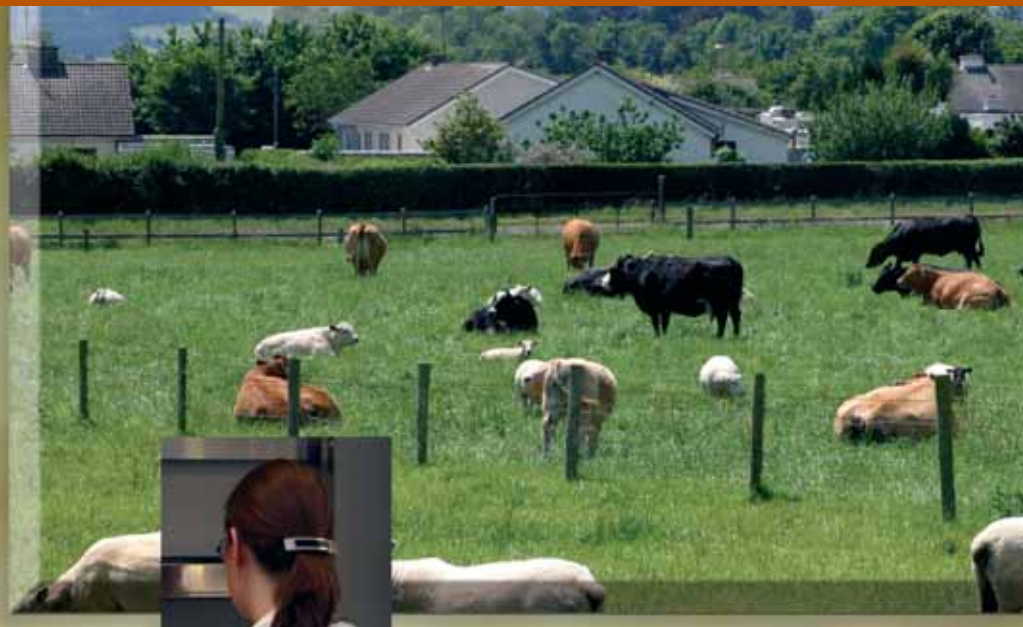
Agriculture and Food Development Authority

FINAL REPORT

Project RMIS No. 5047

The Development and/or Validation of Novel Intervention Technologies to Assure Meat Food Safety



Ashtown Food Research Centre

RESEARCH & TRAINING FOR THE FOOD INDUSTRY

RESEARCH REPORT NO 88

THE DEVELOPMENT AND/OR VALIDATION OF NOVEL INTERVENTION TECHNOLOGIES TO ASSURE MEAT FOOD SAFETY

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This research was funded by the Food Institutional Research Measure (FIRM) administered by the Department of Agriculture and Food except for the carcass sampling methods experiment, which was funded by the Food Safety Authority of Ireland (FSAI).

ISBN 1 84170 478 4
February 2007

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SUMMARY

This project was undertaken to fill some of the knowledge gaps in meat food safety from farm to fork. The data provide the scientific basis for a clean sheep policy to reduce the impact of fleece as a source of microbial contamination on ovine carcasses at the beginning of the slaughter process. At the other end of the slaughter-line, a polyurethane sponge swabbing technology was developed for ovine and bovine carcass sampling as required in 2001/471/EC and the new European Commission Hygiene Regulations. At the processing stages, studies were undertaken to determine the most effective media for the recovery and culture of *Cl. perfringens* cells and spores; the results were then applied to thermal inactivation studies on these bacteria. Thermal resistance data were also obtained for *Bacillus cereus* and a radio frequency cook for meat products was validated in terms of the destruction of *Cl. perfringens* and *B. cereus* cells and spores. Finally, an aerobiology study investigated the effectiveness of a range on measures to prevent air acting as a vector for bacterial dispersion in a meat processing plant.

INTRODUCTION

Meat is an important source of a range of bacterial pathogens including *Salmonella* spp., *E. coli* O157, *Campylobacter* spp. and *Listeria* and represents a significant threat to public health if not produced and processed in a hygienic manner. Meat food safety has therefore been the focus of many research studies and a considerable body of information on most aspects of meat hygiene is currently available in both the scientific and technical literature.

This project initially undertook a review of meat food safety literature currently available and identified significant areas in which data, necessary for the production of microbiologically-safe meat, was lacking. These areas included; [1] data on the relationship between the hygienic status of the ovine fleece and the microbiological status of the resultant carcasses – this is

necessary to provide the scientific basis for a clean sheep policy akin to the clean cattle policy currently implemented by the Department of Agriculture and Food; [2] a sponge swabbing technology equivalent to excision for use in small Irish meat plants; [3] an evaluation of the many different agars currently available for the culturing of *Cl. perfringens* vegetative cells and spores (before and after thermal stress); [4] thermal inactivation data for both *Cl. perfringens* and *B. cereus*; [5] studies on the effect of radio frequency cooking on spore-forming bacterial cells and [6] the role of air as a vector in the contamination of meat products in meat processing plants.

THE DEVELOPMENT OF A CLEAN SHEEP POLICY IN COMPLIANCE WITH HYGIENE REGULATION (EC) 853/2004 [HYGIENE 2]

The aim of this research was to identify the risk factors associated with the transfer of bacterial contamination from the fleece to the ovine carcass thereby providing the scientific basis for the development and validation of a clean sheep policy. Sheep in lairage were graded into five different categories; [A] clean and dry (Figure 1); [B] clean and wet; [C] dirty and dry; [D] dirty and wet (Figure 2) and [E] visible dags (dung-clotted tufts of wool) by the chief veterinary inspector at the slaughter plant based on visual inspection of the fleece. Microbiological evaluations of the carcasses were conducted using swabbing sampling methods. Total viable counts (TVC), total *Enterobacteriaceae* counts (TEC) and total coliform counts (TCC) on the corresponding carcasses were obtained using 40 animals per category at four separate sites (brisket, shoulder, flank and rump) immediately after pelt removal.

Statistical analysis of the TVC data suggested that dirt was a significant factor when the animals were dry. TEC and TCC results suggested that dirt was a contributing risk factor regardless of wetness or dryness of the animal. The clean sheep policy should therefore differentiate between clean and dirty sheep and mandate additional hygiene measures for the latter.

Figure 1. A clean and dry sheep

Figure 2. A dirty and wet sheep

MICROBIOLOGICAL CARCASS SAMPLING METHODS TO ACHIEVE COMPLIANCE WITH 2001/471/EC AND HYGIENE REGULATION (EC) 853/2004 [HYGIENE 2]

The aim of this research was to evaluate the effectiveness of excision versus swabbing as methods for the assessment of bovine and ovine carcass hygiene. Microbiological evaluation of bovine and ovine carcasses was performed by obtaining total viable counts (TVC) and total *Enterobacteriaceae* counts (TEC) using excision and a swab (polyurethane sponge) sampling method. Four anatomical locations were sampled (in accordance with the requirements of regulation 2001/471/EC; Anon 2001) on 30 bovine and 30 ovine carcasses processed in four small (< 10 animals per week) abattoirs. Excision and swab TVC values were similar at all sites (Table 1). The corresponding TEC data were also similar, regardless of sampling method. Swabbing with the polyurethane sponge was therefore as effective as excision sampling for the

determination of TVC and TEC on bovine and ovine carcasses. It was concluded that this sponge swabbing technique may be used instead of excision sampling when assessing bovine and ovine carcass hygiene in accordance with regulation 2001/471/EC and Hygiene regulations.

Table 1. TVC and TEC recovered from bovine and ovine carcasses using excision and swab sampling techniques

Carcass / Carcass site	Type	Excision			Swab		
		Log_{10} cfu cm^{-2}	n	SEM	Log_{10} cfu cm^{-2}	n	SEM
Beef / neck	TVC	2.7	30	0.29	2.1	30	0.28
Beef / brisket	TVC	3.7	30	0.35	3.1	30	0.25
Beef / flank	TVC	3.5	30	0.28	3.4	30	0.30
Beef / rump	TVC	2.7	30	0.25	3.3	30	0.33
Lamb / brisket	TVC	3.9	30	0.28	3.4	30	0.24
Lamb / breast	TVC	4.0	30	0.22	3.4	30	0.19
Lamb / flank	TVC	3.7	30	0.33	3.4	30	0.22
Lamb / thorax	TVC	3.3	30	0.34	3.0	30	0.26
Beef / neck	TEC	0.75	30	0.26	0.83	30	0.20
Beef / brisket	TEC	1.2	30	0.28	1.1	30	0.23
Beef / flank	TEC	0.77	30	0.27	0.61	30	0.18
Beef / rump	TEC	0.53	30	0.25	0.74	30	0.22
Lamb / brisket	TEC	1.1	30	0.29	1.1	30	0.24
Lamb / breast	TEC	1.1	30	0.20	0.77	30	0.19
Lamb / flank	TEC	1.2	30	0.22	1.0	30	0.22
Lamb / thorax	TEC	0.34	30	0.06	0.38	30	0.16

An evaluation of *Clostridium perfringens* media

The objective of this experiment was to test four different agars for suitability as [1] culture media for three different strains of *Cl. perfringens* and [2] recovery media for thermally-treated *Cl. perfringens* spores. The media included [1] tryptose sulphite cycloserine (TSC) with and without egg yoke emulsion (EYE); [2] Shahidi-Ferguson perfringens agar (SFP) with and without EYE; [3] oleandomycin polymyxin sulfadiazine perfringens (OPSP) (pour plate and spread plate) and [4] reinforced clostridial agar (RCA). Three strains of *Cl. perfringens* were used i.e. *Cl. perfringens* DSM 11784, *Cl. perfringens* NCTC 08237 and *Cl. perfringens* NCTC 10614. Optimal growth of *Cl. perfringens* was obtained on RCA and TSC (with or without EYE) regardless of strain (Table 2); growth of thermally-treated spores was only obtained on RCA. RCA should therefore be used when testing foods and water for the presence of *Cl. perfringens* and was used in all relevant studies in this project.

Table 2. Cl. *perfringens* DSM 11784, NCTC 08237 and NCTC 10614 recovered on different media

Medium	DSM 11784	NCTC 08237	NCTC 10614
		Mean (log_{10} cfu ml-1)	
TSC+	7.1	6.50	6.17
TSC-	7.1	6.52	6.79
SFP+	6.2	6.13	6.19
SFP-	6.1	5.51	5.50
OPSP (sp)[1]	6.1	4.39	4.28
OPSP (pp) [2]	6.5	4.30	4.14
RCA	7.0	6.79	6.30
sp[1] = spread plate; pp[2] = pour plate			

Thermal inactivation of *Bacillus cereus* and *Clostridium perfringens* vegetative cells and spores in pork luncheon roll

The aim of this study was to design a thermal treatment(s) for pork luncheon roll which would destroy *Bacillus cereus* and *Clostridium perfringens* vegetative cells and spores. *B. cereus* and *Cl. perfringens* vegetative and spore cocktails were used to inoculate luncheon meat prior to cooking. Samples were subjected to different temperatures and removal times. The decimal-reduction times (D-values) were calculated by linear regression analysis (D = -1/slope of a plot of log surviving cells versus time) and z-values obtained from the plot of $\log_{10}$ D-values against their corresponding temperatures. D-values for vegetative cells ranged from 1 min at 60 °C to 33.2 min at 50 ºC for *B. cereus* and from 0.9 min (65 °C) to 16.3 min (55 °C) for *Cl. perfringens* (Table 3). The D-values for *B. cereus* spores ranged from 2.0 min (95 °C) to 32.1 min (85 °C) and from 2.2 min (100 °C) to 34.2 min (90 ºC) for *Cl. perfringens* (Table 3).The z-values were calculated to be 6.6 C°* and 8.5 C° for *B. cereus* vegetative and spores respectively; corresponding values of 7.8 C° and 8.4 C° for *Cl. perfringens* vegetative cells and spores respectively were also obtained. D-values of *B. cereus* and *Cl. perfringens* suggest that a mild cook of 70 °C for 1.3 min would achieve a 6 log reduction of *B. cereus* and *Cl. perfringens* vegetative cells but a cook of 110 °C for 36 s is required to achieve a 6 log reduction of *B. cereus* and *Cl. perfringens* spores. The results of this study provide the thermal inactivation data necessary to design a cooking protocol for pork luncheon roll which would inactivate *B. cereus* and *Cl. perfringens* vegetative cells and spores. The data may also be used in future risk assessment studies.

*Note C° means difference in temperature (°C)

Table 3. D-values (min) for *Bacillus cereus* and *Clostridium perfringens* vegetative cells and spores at different temperatures in pork luncheon roll

Validation of radio frequency cooking to ensure the destruction of *Bacillus cereus* and *Clostridium perfringens* vegetative cells and spores in pork luncheon roll

B. cereus and *Cl. perfringens* vegetative cells and spore cocktails were used to inoculate pork luncheon meat prior to cooking. The meat was vacuum packed, inserted in a casing and radio frequency (RF) cooked (see Figure 3). The temperature of each luncheon roll was measured at different points within the product and recorded. Cooked pork luncheon meat was aseptically divided into twenty sections with *B. cereus* or *Cl. perfringens* vegetative cells

and spores in each section being enumerated. The cooked pork luncheon roll had a temperature profile ranging between 66.9 °C and 78.6 °C. The RF cooks reduced *B. cereus* vegetative cell and spores by 5.4 and 1.8 $\log_{10}$ cfu ml^{-1} respectively. The corresponding reductions for *Cl. perfringens* vegetative cells and spores were 6.8 and 4.1 $\log_{10}$ cfu ml^{-1} respectively. These results suggest that RF heating inactivates *Cl. perfringens* and *B. cereus* cells and spores. Food manufacturers could use the data reported in this study to design an RF cooking procedure for pork luncheon roll and other similar meat products.

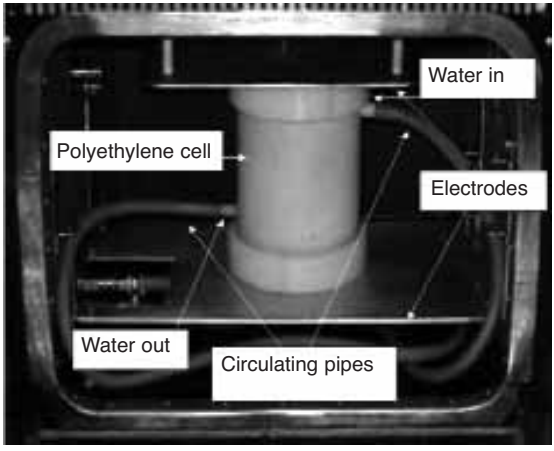

Figure 3. The radiofrequency unit

AN ASSESSMENT OF POTENTIAL RISK FACTORS RELATING TO THE MICROBIAL CONTAMINATION OF AIR IN A PORK PROCESSING PLANT

The aim of this study was to analyse the microbial quality of air throughout a pork burger plant and to identify the primary source of airborne contamination within the plant. The air in the pork processing plant was analysed for total viable bacteria, total coliform count and the presence of *Staphylococcus* at various different locations throughout the day's production. The plant was also audited to establish the factors influencing air quality.

Though the microbial levels recorded were low (see Table 4 for TVC and Table 5 for TCC), this study found that the most heavily contaminated area

in terms of TVC in the production process was the cooking area and the most contaminated time throughout the production day was 12:30pm. However, the audit showed that the 'cooked' area had the same air quality controls as each of the other stages in production (wall separation, no door openings, an effective ventilation system, air flow from clean to dirty areas, closed drains, no aerosol generation, no dusty ingredients, no condensation and an effective cleaning operation) except for the fact that this area had more operatives. The results are therefore inconclusive and a more detailed study is required before specific recommendations can be made.

Table 4. Total viable counts (mean values; cfu/m^3) at various sites in a pork processing plant.

Location	Time					Mean
	5:00AM	9:30AM	12:30PM	4:00PM	7:30PM	
	total viable counts (TVC) cfu/m^3)					
Raw	52	43	12	30	9	29
Pre-cooked	14	21	422	0	5	92
Cooking	429	6	421	3	3	172
Post-cooked	49	13	5	4	7	16
Blast-chill	139	25	13	12	39	46
Packaging	5	9	3	5	3	5
Mean	15	20	146	9	11	60

Table 5. Total coliform counts (mean values; cfu/m^3) at various sites in a pork processing plant.

Location	Processing time					Mean
	5:00AM	9:30AM	12:30PM	4:00PM	7:30PM	
	total viable counts (TVC) cfu/m^3)					
Raw	4	5	1	1	1	2
Pre-cooked	2	7	1	1	0	2
Cooking	3	2	13	3	1	4
Post-cooked	4	7	5	0	1	3
Blast-chill	33	3	1	0	8	9
Packaging	1	0	0	0	1	0
Mean	8	4	4	1	2	4

CONCLUSIONS

The conclusions of this project are as follows:

- The clean sheep policy should simply distinguish between clean and dirty animals. The latter should be slaughtered at the end of the day, at a slower line speed and with additional hygiene measures such as detailed visual inspection of the carcasses and additional trimming where necessary to remove faecal stains.

- The polyurethane sponge swabbing technique may be used instead of excision for bovine and ovine carcass sampling in small meat plants.

- RCA is the most suitable medium for the recovery and culturing of *Cl. perfringens* cells and spores including those subject to a thermal stress.

- Radio frequency cooking will achieve a sufficient reduction in *B. cereus* and *Cl. perfringens* cells to assure the safety of meat products and should be used in association with rapid chilling to prevent spore germination.

- Personnel are a significant source of airborne bacterial contamination in meat processing plants.

REFERENCES

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